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VAPOR PRESSURE AND SURFACE TENSION MEASUREMENTS OF  
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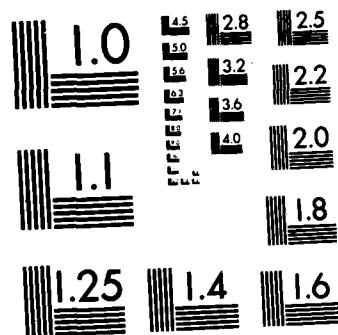
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REPORT DOCUMENTATION PAGE

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1. REPORT NUMBER Technical Note No. 82-41TN	2. GOVT ACCESSION NO. AD-A125443	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Vapor Pressure and Surface Tension Measurements of Seven Mosquito Repellents		5. TYPE OF REPORT & PERIOD COVERED Final Sep 78 - 1 Jan 79
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) June R. Jaeger, BS William G. Reifenrath, PhD Louis C. Rutledge, MS		8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS Division of Cutaneous Hazards Letterman Army Institute of Research Presidio of San Francisco, CA 94129		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 3M161102AS10 WU 250
11. CONTROLLING OFFICE NAME AND ADDRESS		12. REPORT DATE November 1982
		13. NUMBER OF PAGES 13
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		15. SECURITY CLASS. (of this report) Unclassified
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE
16. DISTRIBUTION STATEMENT (of this Report) This document has been approved for public release and sale: Its distribution is unlimited		
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)		
18. SUPPLEMENTARY NOTES		
19. KEY WORDS (Continue on reverse side if necessary and identify by block number) Keywords - 2-ethyl-1,3-hexanediol, N,N-diethyl-m-toluamide, dimethyl phthalate, 1, -(butylsulfonyl) hexanhydro-1H-azepine, N,N-dicyclohexamethyleneurea, triethylene glycol monohexyl ether, butyl 3,4-dihydro-2,2-dimethyl-4-oxo-2H-pyran-6-carboxylate, mosquito repellent relative volatility, surface tension, dosage, evaporation, repellency, insect repellent, Aedes aegypti.		
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**VAPOR PRESSURE AND SURFACE TENSION MEASUREMENTS  
OF SEVEN MOSQUITO REPELLENTS**

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**NOVEMBER 1982**

**LETTERMAN ARMY INSTITUTE OF RESEARCH  
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129**

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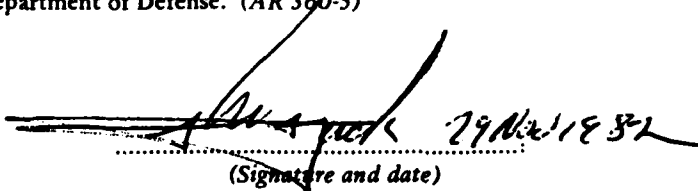
Vapor Pressure and Surface Tension Measurements of Seven Mosquito Repellents  
--Jaeger, Reifenrath, and Rutledge

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# ABSTRACT

An investigation was initiated to determine whether or not a relationship exists, first, between the relative vapor pressure of a repellent and its loss from the skin by evaporation and, second, between the surface tension of a mosquito repellent and its intrinsic repellency. The relative vapor pressure of five of these compounds was measured by a head space sampling gas chromatographic procedure. The surface tension of seven mosquito repellents was measured by a pendant drop or du Nouy method. The median effective dose ( $ED_{50}$ ) of the seven compounds was determined against Aedes aegypti mosquitoes. A comparison was made between repellent  $ED_{50}$  values and the more difficult to measure minimum effective evaporation rate (MEER) or intrinsic repellency of 4 of the compounds. Repellent  $ED_{50}$  increased as MEER increased which supports the concept that  $ED_{50}$  is a measure of intrinsic repellency. A comparison was made between previously reported values for repellent evaporative loss from skin and relative vapor pressure. No simple relationship exists between evaporative loss from the skin surface and relative vapor pressure. No apparent linear relationship exists between surface tension and  $ED_{50}$  values.

## VAPOR PRESSURE AND SURFACE TENSION MEASUREMENTS OF SEVEN MOSQUITO REPELLENTS

The fate of a chemical on the skin surface is dependent in part on the physical and chemical properties of the substance (1). The relative vapor pressure of a chemical may be an important predictor of how much will be lost from the skin by evaporation. In the case of mosquito repellents, evaporation from the skin is not only a mode of loss, but also is an essential property for repellent activity (2,3) and is one determinant of repellent duration (Reifenrath and Robinson, unpublished data).

A wide variety of chemical classes repel mosquitoes (4). This suggests that repellent activity is dependent on physical properties. The surface tension (liquid/air interface) of a repellent may be one of the physical properties. The tendency for a liquid to spread on a surface is dependent on the surface tension of the liquid/air interface, the surface/air interface and the surface/liquid interface. Other things being equal, the higher the surface tension of the liquid/air interface, the greater the tendency for the liquid to occupy the least surface area, which results in reduced wetting of the surface. Mosquito repellents come in contact with at least two different surfaces, the skin of man and the surface of the mosquito, the cuticula. The cuticula/repellent contact initiates the repulsion of the mosquito. If a thin film of repellent results from this contact, the surface tension of the repellent may be important for determining if the repellent/cuticula interaction results in mosquito repulsion. A study was initiated to determine whether a relationship exists, first, between the relative vapor pressure of a repellent and its loss from the skin by evaporation and, second, between the surface tension of a repellent and its intrinsic repellency. The relative vapor pressure values for several repellents were measured and compared to previously reported loss from the skin surface by evaporation. The median effective dose ( $ED_{50}$ ) values of several repellents were determined as a measure of intrinsic repellency and these were compared to measured surface tension values.



## MATERIALS AND METHODS

Compounds. The following mosquito repellent compounds were tested: 2-ethyl-1,3-hexanediol, Niagara Chemical Division, FMC, Middleport, NY; N,N-diethyl-m-toluamide, Aldrich Chemical Co., Milwaukee, WI; dimethyl phthalate, Niagara Chemical Division, FMC, Middleport, NY; 1-(butylsulfonyl)hexahydro-1H-azepine (sulfonamide), N,N'-dicyclohexamethyleneurea (carbamide) and triethylene glycol monohexyl ether (SRI 6), SRI International, Menlo Park, CA; and butyl 3,4-dihydro-2,2-dimethyl-4-oxo-2H-pyran-6-carboxylate (indalone), ICN Pharmaceuticals, K&K Laboratories Division, Plainview, NY. The following standards were used without further purification: n-octanol, reagent grade, Matheson Coleman & Bell, Norwood, Ohio; hexane, spectro quality, Matheson Coleman, & Bell; n-butanol, reagent grade, J.T. Baker Chemical Co, Phillipsburg, NJ.

Relative Vapor Pressure Determination. Closed 5 ml Mininert Reactivials (Pierce Chemical Co., Rockford, IL) equipped with screw caps and valves containing 1.0 ml of each repellent were placed in a 30 C controlled temperature water bath chamber (Lauda WB-20/R, Brinkman Instruments) and left to equilibrate for 2 hr. Repetitive sampling of the vapor phase was done by inserting the needle attached to a 100  $\mu$ l Pressure-Lok, Series A-2, gas syringe (Supelco, Bellafonte, PA) equipped with a push button valve, into the headspace of the vial. It was left in the 0  $\mu$ l position for 4 min, then the plunger was withdrawn to the 100  $\mu$ l mark. After an exact interval of 60 sec the needle was withdrawn and the valve on the barrel closed. The plunger was then advanced to the 10  $\mu$ l mark to compress the vapors and the needle inserted through the septum of the gas chromatograph (Perkin Elmer, Model 900) at which time the valve on the barrel was opened and the plunger on the syringe was depressed to the 0  $\mu$ l mark. Gas liquid chromatography conditions: A 6 ft (1.8 m), 2 mm inside diameter, coiled glass column packed with 3% OV-17 on Gas Chrom Q 100/120 mesh (Supelco) was used. The column oven was set at 100 C for 2-ethyl-1,3-hexanediol (compound I), 120 C for N,N-diethyl-m-toluamide (compound II) and dimethyl phthalate (compound III), 160 C for sulfonamide (compound IV) and 180 C for carbamide (compound V). The injector temperature was set at 250 C and the detector at 300 C for all compounds. The flow rate of carrier gas helium at the column exit was 26 ml/min for compound I and 40 ml/min for compounds II through V. The area under the recorded peak for each compound was determined by making a photocopy of the chromatogram, then cutting out the area under the curve and weighing it on an analytical balance. The weight was translated into mass units by reference to a standard curve plot of the mass of a known liquid volume of the same compound injected versus the peak area determined by using the same photocopy-cut-weigh procedure.

The vapor pressure of a compound,  $VP_c$ , at a given temperature was determined by the following equation:

$$VP_c = \frac{n_c \times 760 \text{ mm}}{n}$$

where  $n_c$  is the number of moles of repellent compound in  $3.5 \text{ cm}^3$  headspace volume of the vial and  $n$  is the total number of moles of molecules in the  $3.5 \text{ cm}^3$  volume. The value of  $n$  is determined from the following equation:

$$n = \frac{PV}{RT}$$

where  $P$  is the pressure (assumed to be 1 atmosphere),  $V$  is volume ( $3.5 \text{ cm}^3$ ),  $R$  is the gas constant (82.856) and  $T$  is the temperature in degrees Kelvin of the water bath.

Surface Tension Determination. Surface tension was measured by the pendant drop method (5) for six repellent compounds. The upper end of a vertically mounted capillary tube (inside diameter = 0.3 mm, outside diameter = 5.5 mm, length = 15 cm) was attached to a reservoir for containment of the liquid under study. The reservoir was filled to such a level as to provide a slow drip rate from the other end of the capillary tube (approximately 1 drop per 30 sec). A metric scale was attached parallel to the length of the capillary tube. A Mitchell camera, equipped with a 100 mm f2.8 lens, was positioned perpendicularly to the plane of the metric scale and focused on the lower end of the capillary tube. The capillary tube assembly was illuminated directly with light at a 45 degree angle to the line of sight between the lower end of the capillary tube and the center of the lens and indirectly by light reflected from a white background. The camera was operated at 96 frames per second on Tri-X film. Film was developed by Versamat processing. Surface tension was calculated from the equation:

$$\gamma = \frac{g \sigma d_e^2}{H}$$

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\* The actual volume of an empty 5 ml Mininert Reactivial was measured with a graduated pipet and found to contain 4.5 ml. After 1.0 ml of repellent compound was added to the vial,  $3.5 \text{ mm}^3$  of headspace volume remained.

where  $\gamma$  is the surface tension,  $g$  is the acceleration due to gravity ( $980.621 \text{ cm/sec}^2$ ),  $\sigma$  is the density of the liquid under study,  $d_e$  is the maximum diameter of the pendant drop and  $H$  (a drop shape parameter) is a function of the parameter  $S$ .

$$S = \frac{d_s}{d_e}$$

where  $d_s$  is the diameter of the drop at a vertical distance  $d_s$  from the bottom of the pendant drop. Values of  $d_s$  and  $d_e$  were determined from photographic measurements of the drop in various stages.  $S$  was then calculated and  $H$  determined from published tables of  $1/H$  in terms of  $S$  (5). For a given compound,  $\gamma$  versus  $S$  was plotted for values of  $S$  greater than 0.85. Linear plots resulted and the value of  $\gamma$  obtained by extrapolating the line to  $S = 1.00$  was taken as the surface tension. Single determinations were made.

For 2-ethyl-1,-hexanediol, the du Nouy method of surface tension measurement was used. A Fisher Autotensiomat (Fisher Scientific Co, Pittsburg, PA) was used employing standard procedures. The mean of 3 separate trials was determined.

Median Effective Dosage Determinations. The  $ED_{50}$ s of the test materials for the yellow fever mosquito, *Aedes aegypti* L., were determined by the method of Rutledge et al (6).

Minimum Effective Evaporation Rate (MEER) Determinations. The MEER for a given repellent was determined by applying the compound's minimum effective dose (against *Aedes aegypti* (7)) to excised skin and measuring the amount of evaporation over a 1-hour period of time (8). Values were determined from 3 separate replicates.

Twelve Hour In Vitro Evaporation Determinations. Twelve-hour in vitro evaporation from skin was measured by applying the repellent at a dose of  $0.3 \text{ mg/cm}^2$  to excised skin and trapping the vapor over a 12 hour period (8).

## RESULTS

The concentration of compound in the vapor phase versus temperature for several repellents is given in Figure 1. Table 1 contains the repellent vapor pressure calculated at 30 C. Table 2 contains the surface tension of various repellents and standards.

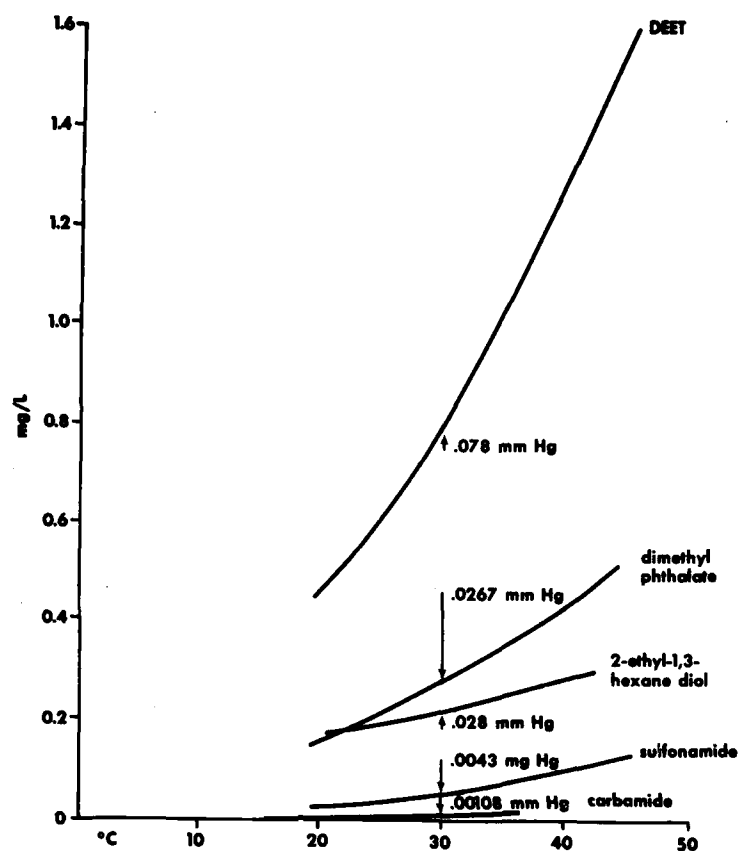


Figure 1. Concentrations in the vapor phase (mg/L) of pure repellents at various temperatures.

Table 1. Relative vapor pressure and evaporative loss of repellents

Compound	Relative vapor pressure	
	(mm Hg at 30 °C)*	12-hour in vitro evaporation from skin (percent of applied dose)+
2-Ethyl-1,3-hexanediol	0.028	47.2+4.3
N,N-diethyl-m-toluamide	0.078	16.7+3.7
Dimethyl phthalate	0.027	...
Sulfonamide	0.0043	6.2+1.6
Carbamide	0.0011	3.0+2.6

\* Mean of duplicate determinations

+ Topical dose of 0.3 mg/cm<sup>2</sup>

Table 2. Surface tension of various compounds and median effective dose (ED<sub>50</sub>) and minimum effective evaporation rate (MEER) of various repellents against Aedes aegypti mosquitoes.

Compound	Surface tension (dynes/cm <sup>2</sup> , 20 C)	ED <sub>50</sub> (mg/cm <sup>2</sup> )*	MEER (ug/cm <sup>2</sup> /hr) <sup>+</sup>
Water	70.6(72.7)‡	...	...
Dimethyl phthalate	44.2	0.066 (0.047-.097)	...
Sulfonamide	42.1	0.005 (.001-.013)	0.20±0.09
Carbamide	41.2	0.014 (.010-.019)	1.1±0.1
N,N-diethyl-m-toluamide	39.2	0.029 (.010-.045)	1.2±0.3
Indalone	35.0	0.031 (.027-.035)	...
SRI-6	34.8	0.062 (.056-.060)	...
2-Ethyl-1,3-hexanediol	32.1	0.129 (.080-.452)	7.5±1.7
n-Octanol	24.3(27.5)‡	...	...
n-Butanol	21.6(24.6)‡	...	...
Hexane	19.4(18.4)‡	...	...

\* 95% confidence limits in parenthesis.

<sup>+</sup>The lower the value, the more potent the compound in repelling Aedes aegypti mosquitoes.

‡Literature values in parentheses from CRC Handbook of Chemistry and Physics, 45th Edition, 1964-1965. These compounds are not insect repellents.

## DISCUSSION

The vapor pressures measured are relative values, dependent on the method of sampling. Reproducible results can be obtained only by precisely following the procedure. The technique does provide a numerical method of comparison of one compound with another and a way of ranking groups of compounds according to their vapor pressure. The data in Table 1 provide some meaning of the impact of magnitude changes in vapor pressure on evaporative loss from the skin, (compare 2-ethyl-1,3-hexanediol and N,N-diethyl-m-toluamide with sulfonamide and carbonate) but there is no simple relationship between relative vapor pressure and evaporative losses from the skin surface (eg. compare 2-ethyl-1,3-hexanediol with N,N-diethyl-m-toluamide). Chemicals with similar vapor pressures could have different evaporative losses from the skin surface due to different interactions with the skin.

The method we employed for measurement of surface tension gave comparable results for those compounds whose values were previously reported (Table 2). Table 2 lists available median effective dose ( $ED_{50}$ ) values and minimum effective evaporation rate (MEER) values for repellents against Aedes aegypti mosquitoes. MEER values are measures of the intrinsic repellency of a compound in repelling Aedes aegypti mosquitoes under given test conditions. Although data for comparison are limited, the available  $ED_{50}$  determinations rank the potency of repellents in the same order as MEER determinations. No apparent linear relationship exists between surface tension and  $ED_{50}$  values (Table 2).

## CONCLUSION

No simple relationship exists between relative vapor pressure and how much of a compound might be lost from the skin surface by evaporation. Other factors such as skin absorption will also affect the disposition of chemicals on the skin.

## RECOMMENDATIONS

Further comparisons of relative vapor pressure and evaporation of repellents from the skin should be made. These determinations should be combined with other measurements of skin interaction of chemicals to provide better estimates of a compound's loss from the skin surface. Although no apparent linear relationship exists between surface tension and  $ED_{50}$  values, it may be that an optimal surface tension near that for sulfonamide is required for maximum intrinsic repellency. Further testing is necessary to substantiate this hypothesis.

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